5. (Amended) The method of claim 4 wherein said primers are selected from the group consisting of those primers set forth in Figure 6 (SEQ ID NOS: 3-42).

REMARKS

As an initial matter, Applicants gratefully acknowledge the many helpful comments found throughout the instant Action. The Examiner's time in preparing the comments is much appreciated.

Support for the present amendments can be found throughout the application including the claims and Drawings as filed originally. No new matter has been added by virtue of the amendments.

On pgs. 2-3 of the Action, claim 1 was objected to. Although Applicants must respectfully disagree with the Office as to the need for the suggested language, that language has been adopted in amended claim 1 in the interest of furthering progress in this case.

On pg. 3, the position was taken that the cloning or amplifying step, as recited, was confusing. Applicants respectfully disagree and point out that the Examiner's understanding of the step is correct.

Claim 1 was objected to on grounds that the phrase "selected from" should be "selected from the group consisting of". The objection has been addressed by adopting the Examiner's suggested claim language.

Claim 1 was further objected to on grounds that the "recovering" step "does not result in isolation of a nucleic acid molecule encoding a TAA-specific TCR as in the preamble." Office Action at 3. Although Applicants respectfully disagree with the position taken, grounds for it have been addressed by the present amendment to claim 1.

At pgs. 3-4, the specification was objected to on various grounds. Although Applicants respectfully disagree, the objections have been addressed by this submission.

Rejection under 35 USC §112, first paragraph

Claims 1-5 stand rejected under 35 USC §112 ,first paragraph, as not being enabling for preparing certain nucleic acids encoding non-human HLA-restricted TCRs specific for TAA. Although Applicants respectfully disagree, claim 1 has been amended to mouse or transgenic mice to further prosecution of this application.

Claim 1 was further rejected on grounds that Applicants have not claimed a mouse TCR. Applicants disagree that such specific language should be required in this case. However in the interest of furthering prosecution, claim 1 has been amended to recite mouse TCR as requested by the Examiner.

Claim 1 also stands rejected on grounds that "without adequate levels of HLA expression and production of CTL that recognize the administered antigen, **the transgenic non-human mammal claimed** is of no use." Action at pg. 6 (emphasis added). Applicants must disagree.

First, it is respectfully pointed out that claim 1 features a method and not a transgenic non-human mammal. Applicants are not claiming any transgenic mammals at this time.

Moreover, one of skill having read the instant disclosure and particularly claim 1 (as presently amended) would understand that CTL recognition, as recited in the claimed method, would be difficult, if not impossible, without adequate APC expression of the human HLA protein. In the unlikely event that a cell expresses "only one human HLA molecule", as alleged in the Action, CTL recognition of the TAA would be negligible. Also, it would be difficult to obtain the TAA-specific, HLA-restricted CTL of the claimed method in the absence of adequate expression levels.

Any inoperable embodiments of the type described by the rejection could be readily avoided. As described by the Court of Customs and Appeals:

> [M]any patented claims read on vast numbers of inoperative embodiments in the trivial sense that they can and do omit factors which must be presumed to be within the level of ordinary skill in the art.' ... There is nothing wrong with this so long as it would be obvious to one of skill in the art how to include these factors in such manner as to make the embodiment operative rather than inoperative. *In re Cook and Merigold*, 169 USPQ 299, 302 (C.C.P.A. 1971) (quoting *In re Skrivan*, 166 USPQ 85, 88 (C.C.P.A. 1970)).

It is urged that one of skill having read Applicants' disclosure would know how to identify adequate APC expression of human HLA eg., by obtaining CTL that recognize TAA (followed by activation). Even if one assumes, *arguendo*, that a particular invention embodiment "expressed only one HLA molecule", as alleged, that result, by itself, would not support the present enablement rejection. The worker would understand that adequate APC expression of human HLA could be achieved by other means provided by the specification. See, for instance, Examples 1 and 2 (describing how to obtain and use CTL obtained from transgenic mice).

Thus, Applicants see no reason why they should be required to add language about adequate levels of APC expression of human HLA. Such information is clearly not needed for one to understand how to make and use the invention as claimed. Reconsideration and withdrawal of this ground of rejection are requested.

On pg. 7 of the Action, claim 7 stands rejected on grounds that the specification only teaches transgenic mice expressing HLA-A2. Although Applicants respectfully disagree, the claim has been amended as suggested by the Examiner to further prosecution.

Claim 1 was further rejected on grounds that use of the p53 antigen in the method will not work ie., "either p53 is not expressed on the surface of the tumor or the p53 protein is not expressed to adequate levels such that a CTL response against p53 is produced (cit. omitted)."

While Applicants must respectfully disagree with the position taken, grounds for it have been addressed by this submission.

Rejections under 35 USC §112, second paragraph

Claims 1-5 stand rejected on grounds of being indefinite. The Examiner's concerns are addressed in the order in which they appear in the Action.

The position was taken that claim 1 is indefinite because the recovering step does not result in isolation of a nucleic acid molecule encoding a TAA-specific TCR as provided in the preamble. Although Applicants respectfully disagree, basis for the rejection has been addressed. Specifically, amended claim 1 features an isolation step.

Claim 1 stands rejected as indefinite on grounds that the phrase "encoding at least one of the variable regions of the α and β chains". Action at 10. Although Applicants disagree that the phrase is indefinite, basis for the rejection has been addressed by this submission. Claim 1 has been amended to clarify that the recited nucleic acid molecule has a nucleotide sequence encoding at least one of the variable regions of each of the α and β chains. As written then, the nucleotide sequence can encode one or both of the chains.

On pg. 11 of the Action, claim 4 stands rejected as being indefinite. While Applicants disagree, basis for the rejection has been addressed by this submission.

Also on pg. 11, claim 5 stands rejected as being indefinite. Applicants respectfully disagree. However, grounds for the rejection has been addressed by this submission.

Claims 1-5 stand rejected as being obvious under 35 USC §103 in view of Man et al. (1994) *J. Immunol.* 153: 4458) and Cole et al. (1995) *FASEB Journal* 9: A801, abstract 4638). Applicants respectfully disagree with the rejection, particularly in view of the present amendments.

The position is taken in the rejection that Man teaches immunizing transgenic mice expressing a human MHC I complex (HLA-A2.1) with influenza A antigen ($M1_{58-66}$), isolating cytotoxic T lymphocytes (CTL) from the mice that lyse the M1, and then amplifying nucleic acid encoding TCR from the CTL. Action at 11. The Office has acknowledged that Man does not show how to use the transgenic mouse to isolate tumor associated antigen (TAA)-specific TCR. Action at 11. Cole is used in attempt to remedy this deficiency. Specifically, the Cole reference is relied on as providing that CTL can recognize MART-1 in an HLA-A2 restricted manner and that the CTL can be employed to isolate corresponding TCR. It is alleged that it would be obvious to replace the influenza M1 antigen with the MART-1 antigen to obtain MART-1 specific TCR in vivo. Respectfully, the rejection cannot stand scrutiny.

There is nothing in the cited references, taken together or individually, that provides any specific teaching, motivation or suggestion to substitute Man's influenza M1 antigen with Cole's MART-1 antigen. Quite to the contrary, one of skill in this particular field would have been dissuaded from attempting such an experiment in view of what Man discloses:

> Another factor to consider is that **selection of murine TCR on HLA-A2.1 in these transgenic mice is inefficient** when compared with the selection on the endogenous mouse MHC molecules (cites omitted).
>
> See Man et al. at pg. 4465, col. 2 (emphasis added).

Thus at least in Man's hands, it was difficult to select murine TCR using HLA-A2 transgenic mice because the process was reported to be inefficient. There is no expressed teaching, suggestion or motivation in the cited references that would lead one to compound the supposed inefficiency by substituting Cole's untried MART-1 antigen in Man's assay.

The Man reference also casts substantial doubt on the ability of human HLA-A2 transgenic mice to provide CTL. According to Man:

> It is possible that **only a proportion of peripheral T cells** from HLA-A2.1 transgenic mice **have actually been selected** on HLA-A2.1 as opposed to murine molecules. The

difference in efficiency of selection is likely to reflect the **suboptimal interaction** between murine CD8 and α3 domain of the HLA molecule (cites omitted).

Thus according to the Man reference, the human HLA-A2.1 molecule, when in the context of the transgenic mouse, does not interact well with murine CD8. Because there is understanding in the field that CD8 is helpful in obtaining good T cell recognition, Man's warning would further dissuade one from attempting the experiment. More particularly, a worker would view the shortcoming discussed by Man as harming ability to isolate suitable levels of CTL. There is no expressed teaching, suggestion or motivation in the cited references that would lead one to complicate the CD8 interaction problems reported by Man by substituting Cole's untested MART-1 antigen for the M1 antigen taught by Man.

The Cole reference, as cited, does not provide a remedy for these deficiencies. As relied on, Cole merely reports that **cloned CTL lines grown *in vitro*** can be used to isolate MART specific TCRs. There is no expressed teaching or suggestion in Cole that MART specific (or any other TAA) could be recovered from CTL obtained ***in vivo***, particularly from a transgenic mouse expressing the human HLA-A2 molecule.

In marked contrast to the cited combination of references, Applicants have shown for the first time that it is possible to use transgenic mice encoding human HLA-A2 as a good source of CTLs that encode TCR molecules specific for tumor antigens. See the specification eg., at pgs 1-2, bridging paragraph, Examples 1-3 and the Drawings.

Applicants believe that all outstanding grounds of rejection have been addressed. Early consideration and allowance of the application are respectfully requested.

Attached to this submission is a marked-up version of the changes made to the specification and claims. The attached page is captioned "version with markings to show changes made".

If for any reason an additional fee is required, a fee paid is inadequate or credit is owed for any excess fee paid, you are hereby authorized and requested to charge Deposit Account No. **04-1105**.

Respectfully submitted,

Date: October 10, 2001

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE SPECIFICATION:

Page 8, line 3, "reference" has been deleted.

Page 4, lines 1-3 have been deleted and the following paragraph has been added.

Figure 3A and 3B show the complete nucleotide (SEQ ID NO: 1) and deduced amino acid sequence (SEQ ID NO: 2) of a single chain TCR derivative which contains variable α and β specific for HA linked through a short peptide linker and then fused through a CD8 hinge to the ζ chain.

Figure 6 has been deleted and replaced with the attached and amended Figure 6.

IN THE CLAIMS:

Claims 1, 4, and 5 have been amended as follows:

1. (Amended) A method of isolating a ~~to prepare an isolated~~ nucleic acid molecule having a nucleotide sequence encoding at least one of the variable regions of each of the α and β chains of a ~~non-human~~ mouse T-cell receptor (TCR) which TCR is specific for a tumor-associated antigen (TAA) selected from the group consisting of Her-2/neu, RAS, [p53,] tyrosinase, MART, Gp100, Mage, Bage and MUC-1, which method comprises

~~immunizing a transgenic non-human mammal species, which produce human HLA, with an effective amount of said TAA to produce HLA restricted cytotoxic T lymphocytes (CTL) which display TCR specific for said TAA in amounts sufficient to lyse tumor cells having the TAA,~~

immunizing a transgenic mouse whose genome comprises a nucleic acid sequence encoding a human leukocyte antigen (HLA-A2) operatively linked to a promoter, wherein said transgenic mouse expresses the HLA-A2 on the surface of antigen presenting cells (APC), with a tumor associated antigen (TAA) such that the TAA is recognized by cytotoxic T lymphocytes (CTL) of the transgenic mouse and such that TAA-specific, HLA-A2 restricted CTL are obtained,

recovering said ~~HLA~~ HLA-A2 restricted CTL, which contain said nucleic acid molecules encoding at least one of each of the variable regions of the α and β chains of a non-human TCR,

cloning or amplifying said nucleic acid molecule encoding the TCR nucleotide sequence isolated from the ~~HLA~~ HLA-A2 restricted CTL and;

isolating the nucleic acid sequence encoding at least one of the α and β chains of the variable region of the mouse TCR that is specific for the TAA ~~recovering said receptor-encoding nucleic acid molecules~~.

4. (Amended) The method of claim 3 wherein the cloning or amplifying step further comprises a polymerase chain reaction using primers derived from murine TCR is used to amplify said nucleic acid molecule.

5. (Amended) The method of claim 4 wherein said primers are selected from the group consisting of those primers set forth in Figure 6 (SEQ ID NOS: 3-42).

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